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## **SUPPLEMENTARY ONLINE MATERIAL**

### **SEN3-mediated deSUMOylation of Drp1 facilitates interaction with Mff to promote cell death**

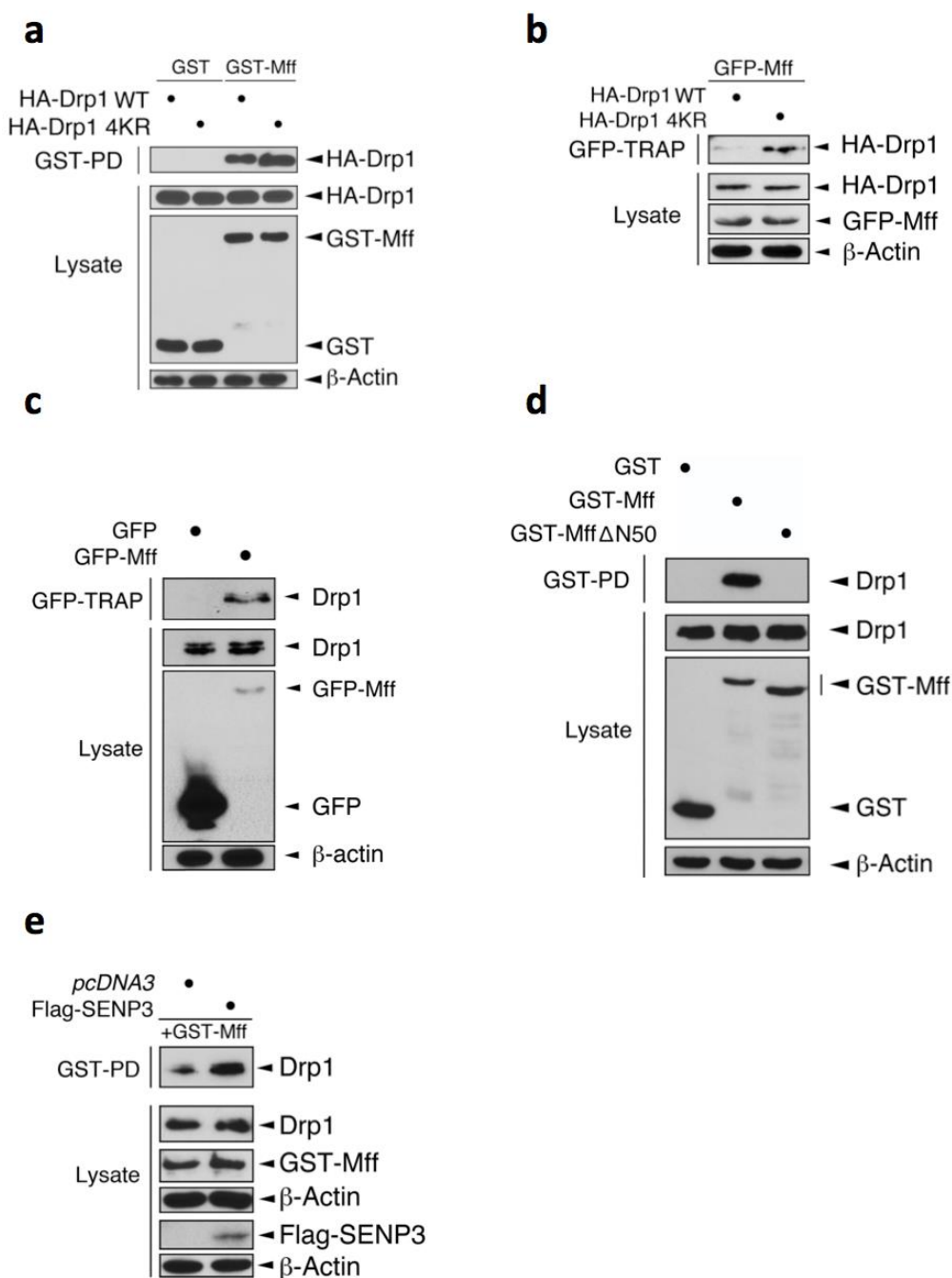
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[This supplement contains:](#)

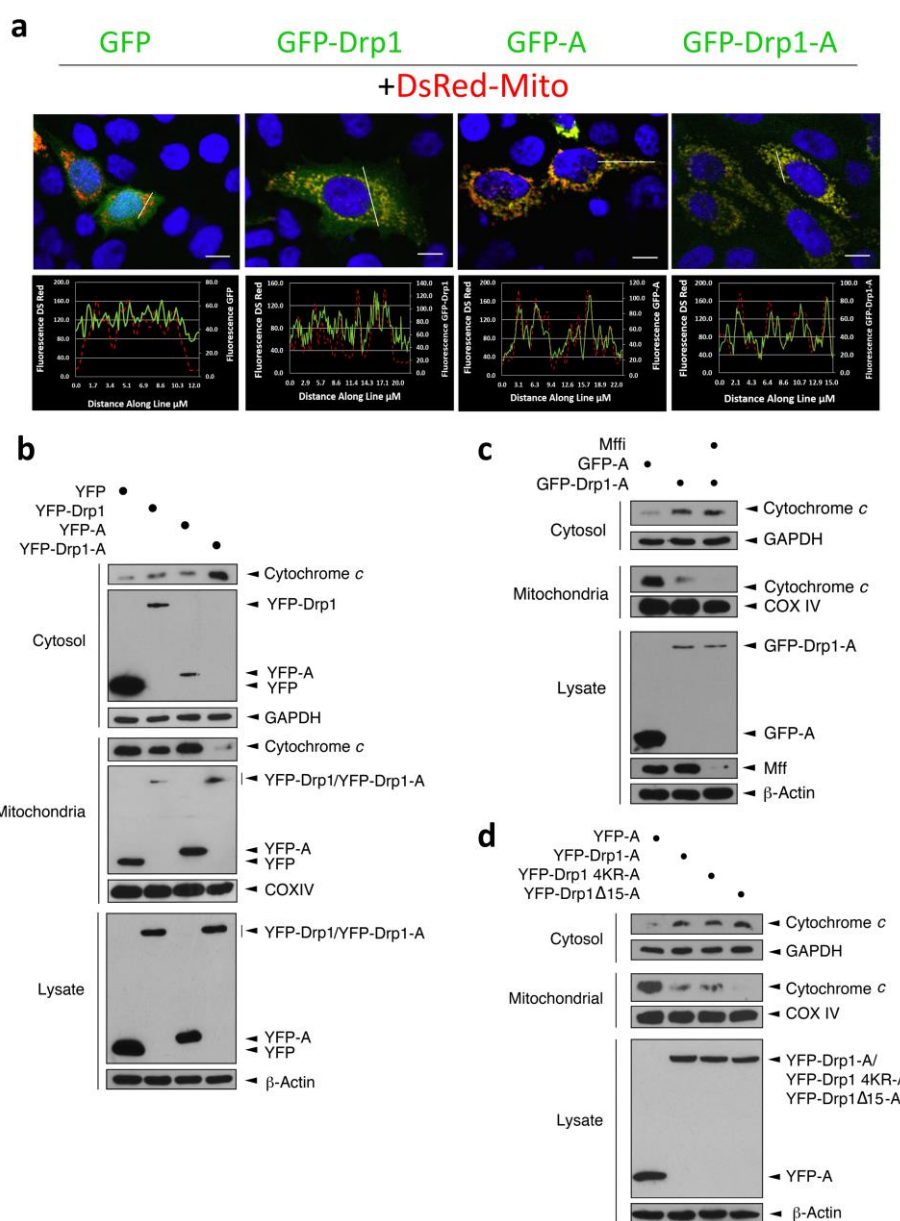
[3 Supplementary Figures](#)



**Supplementary Figure 1** DeSUMOylation of Drp1 promotes its binding to Mff.

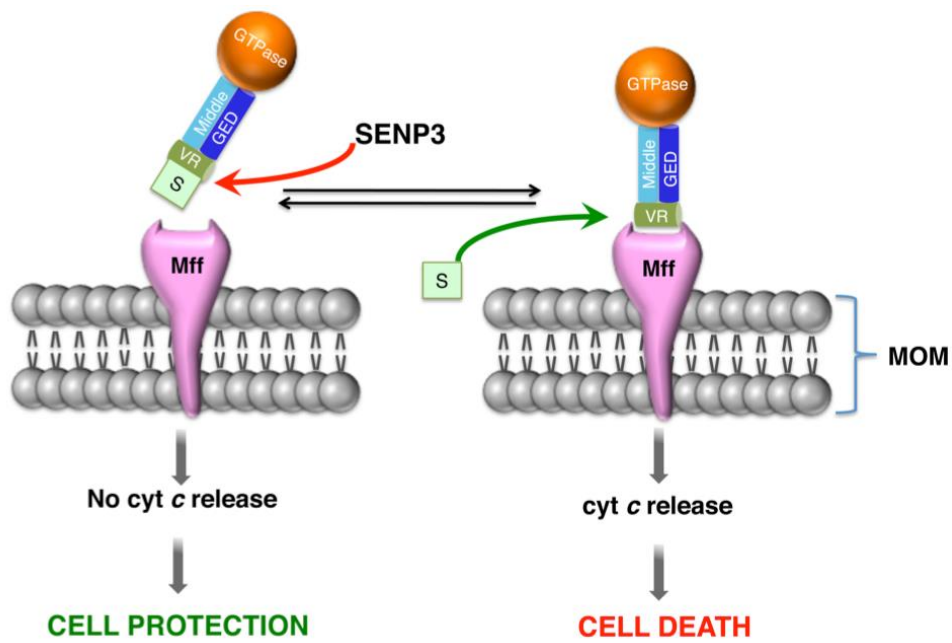
(a) Non-SUMOylatable HA-Drp1 shows enhanced association with GST-Mff in HEK293 cells. Full blots of data shown in **Fig. 1a**. (b) Non-SUMOylatable HA-Drp1 shows enhanced association with GFP-Mff in HEK293 cells. GFP-Mff was pulled down using GFP-TRAP and blotted as shown. (c) Overexpressed GFP-Mff interacts with endogenous Drp1. GFP-TRAP pulldowns were blotted as shown. (d) GST-Mff ΔN50 does not bind Drp1 in HEK293 cells. GST-pulldowns were blotted as shown. (e) Overexpression of SENP3 increases the Mff-Drp1 interaction. Flag-SEN3 was

transfected into HEK293 cells expressing GST-Mff. GST-pulldown and lysate samples were immunoblotted as shown.



**Supplementary Figure 2** Expressing Drp1-A induces Cytochrome c release in HEK293 cells.

(a) Co-localization analysis of GFP, GFP-Drp1, GFP-A, or GFP-Drp1-A with DsRed-Mito (further details for **Fig. 4a**). (b) Subcellular distribution of YFP, YFP-Drp1, YFP-A, or YFP-Drp1-A in HEK293 cells (complete version of **Fig. 4b**). (c) YFP-Drp1-A-induced cytochrome c release bypasses Mff (complete version of **Fig. 4c**). (d) Tethering Drp1 at mitochondria induces cytochrome c release independent of the SUMOylation status of Drp1 (complete version of **Fig. 4d**).



**Supplementary Figure 3** Schematic illustrating how SUMOylation prevents Drp1 interaction with Mff at the MOM.

SENP3 deSUMOylates Drp1, increasing the ability of Drp1 to bind Mff, which leads to cytochrome *c* release. S, SUMO-2/3.